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ACCURACY AND TRACE ORGANIC ANALYSES

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Accuracy in trace organic analysis presents a formidable problem to the residue chemist. He is confronted with the analysis of a large number and variety of compounds present in a multiplicity of substrates at levels as low as parts-per-trillion. At these levels, collection, isolation, identification and quantification are all very difficult. Sample contamination and substrate interferences can also lead to large errors. Obtaining accurate qualitative data is often more of a problem than accuracy of quantitative data. Retention times and peak height measurements from gas chromatography coupled with highly sensitive, but nonspecific, detectors are most commonly used in residue analysis. Although dual column and/or dual detector determination, partition values and chemical derivatization are often employed, lack of good reference standards, interferences and poor detector specificity frequently cast doubt on the qualitative and quantitative accuracy of data upon which regulatory decisions may be made. Mass spectrometry and Fourier transform spectrophotometry offer partial solutions to qualitative accuracy where this instrumentation is available. However, less expensive and more sensitive specific detectors for gas chromatography are most needed. Means of quantitating residues from such complex industrial mixtures as polychlorobiphenyls and toxaphenes are far from adequate. Finally, collection systems for environmental media often lack efficiency, especially for volatile organic compounds in air.

Keywords: Concentration; detectors; extraction; gas chromatography; isolation and clean-up; mass spectrometry; organic trace analysis; qualitative accuracy; quality control; quantitative accuracy; reference materials; sampling.

I. Introduction

When one assesses accuracy, he tends to think in terms of how close he has come to the bullseye, or even to the target as is often the case. In trace

organic analysis, perhaps the more important consideration is whether he aimed at the right target. In the determination of complex organic compounds present at trace or ultratrace levels in our environment, accurate qualitative results are very often more difficult to obtain than accurate quantitative measurements.

The organic analytical chemist is confronted with an almost infinite number of chemical species encompassing a broad spectrum of structure and functionality. In environmental analysis, he must be concerned with their determination at concentrations down to the part-per-trillion level in a multiplicity of media ranging from air to human adipose tissue. It has been estimated that at least 10^5 organic compounds can be expected to be present in a sample of air at a concentration level of one part-per-trillion [1]. Even greater numbers may be contained in other media. The analyst must successfully isolate, concentrate, purify and often derivatize any of these compounds before he can first identify it and finally quantify it. In both of these determinative steps, the need for accuracy is becoming increasingly important. The need for qualitative accuracy probably outweighs the need for quantitative accuracy in organic residue analyses.

A. PESTICIDE RESIDUE ANALYSIS

Work in the field of pesticide residue chemistry has done much toward the development of the sensitive methodologies required for trace organic analyses. Pesticides are probably the most important group of organic chemicals which are deliberately introduced into the environment. Over a billion pounds of organic pesticides are manufactured in the United States yearly [2]. More than 800 different and often very complex chemical compounds are in use as pesticides. They are ultimately dissipated throughout the biosphere to the extent that their residues are found in everything from the air we breathe to our own body tissues.

In the early days of pesticide residue analysis (before about 1965), the chemist depended largely on column and thin-layer chromatography and colorimetry, which were sensitive only to the microgram level. Neither quantitative nor qualitative accuracy were easy to achieve. With the impetus provided by public appeals such as that generated by Rachel Carson's *Silent Spring* [3], a great deal of effort was expended during the 1960's toward the development of more highly sensitive and specific analytical methodologies for pesticides. Building primarily on developments in gas chromatography and improving isolation and clean-up procedures for multi-class compounds, the residue chemist has realized a

millionfold increase in his ability to determine organic pesticides and related compounds. While qualitative accuracy has improved significantly, quantitative accuracy has by no means kept pace with improvements in detection.

B. GAS CHROMATOGRAPHY

Most trace organic analyses today involve gas chromatography (g.c.). Pesticide residue chemistry has played a major role in the development of that field. Many of the most valuable g.c. detectors were devised for determination of pesticides. The most widely used g.c. detectors today are flame ionization, electron capture (or affinity), thermal conductivity, flame photometric, thermionic (or alkali flame ionization) and electrolytic conductivity. The sensitivities and specificities of these detectors are given in table 1. Despite the fact that they offer little or no specificity, the flame ionization and electron capture detectors are most frequently used in trace organic analysis because of the high degree of sensitivity required. The flame photometric detector is more reliable than the thermionic detector, although less sensitive, and is heavily used for determination of organophosphorous and organosulfur compounds. In the phosphorous mode (526 nm filter), its sensitivity for organophosphate pesticides is usually adequate for most residue applications. The electrolytic conductivity detector developed by Coulson [4] has good specificity for chlorine-, sulfur- and nitrogen-containing organic compounds, but lacks the sensitivity necessary for trace analysis. A highly sensitive, specific detector for organonitrogen compounds is badly needed due to the growing

TABLE 1. Gas chromatography detectors

Detector	Specificity	Sensitivity (g)	Linear range*
Thermal conductivity	Nonspecific	10^{-8}	10^5
Flame ionization	Nonspecific	10^{-11}	10^7
Electron capture	Semispecific	10^{-13}	50 to 100
Alkali flame ionization	Specific for P, S, X, N	10^{-12} (for P)	10^3
Flame photometric	Specific for P, S	10^{-10} (for P)	None
Electrolytic conductivity (Coulson)	Specific for X, S, N	10^{-9}	10^3

* Reference: C. H. Hartman, Anal. Chem. **43**, 113A (1971).

markets for the many new carbamate pesticides, which may ultimately replace the more environmentally persistent organochlorine pesticides. One solution to this problem may be the new microelectrolytic conductivity detector devised by Hall [5] with which reportedly as little as 10 pg of nitrogen can be detected. Much hope is also held for its utility as a specific detector for organochlorine pesticides with a sensitivity approaching that of the electron capture detector. At present, however, only the electron capture detector, which has poor specificity, and the essentially universal flame ionization detector afford the sensitivities demanded for the determination of such organic pollutants as pesticides, polychlorobiphenyls, phthalates and vinyl chloride which must be monitored at the parts-per-billion or parts-per-trillion levels. Accuracy of qualitative data, therefore, still remains the primary challenge to the environmental chemist.

C. THE ANALYTICAL SCHEME

The analysis of environmental or biological samples for trace amounts of organic contaminants seldom can be performed directly because of the multitude of interfering substances present in the matrix. Therefore, rather lengthy, multi-step procedures usually are required before the determinative step can be reached. It is through this process that most quantitative errors are introduced.

A typical analytical scheme for the determination of trace organic residues follows these basic steps: 1. extraction, 2. isolation and clean-up, 3. concentration, 4. identification, and 5. measurement.

In order to remove the compound of interest (usually along with a myriad of other compounds) from the matrix in which it is present (tissue, soil, water, sampling media), initial extraction with an organic solvent usually is required. Liquid-liquid partitioning and Soxhlet extraction are employed most often [7]. Complete extraction seldom is achieved. Animal tissues and soils present a large problem for extraction of organic and organometallic compounds. Binding to or solubility in the substrate may be so great as to result in poor extraction efficiencies. Furthermore, these substrates are particularly troublesome for yielding extractable material which interferes with electron capture detection. Isolation and clean-up of the compound is, therefore, mandatory. This may involve column or gel permeation chromatography [6], both of which may result in further losses due to irreversible column adsorption.

After chromatographic clean-up, as well as after extraction, the compound being sought is contained in a relatively large amount of solvent

(and possibly in more than one fraction). This solution must be concentrated before the sample is introduced into the determinative instrument for identification and measurement. Concentration (perhaps by a factor of 10,000 or more) not only results in further loss of the compound, but increases the possibility of interferences from the solvent itself. Loss by volatilization may be severe, especially for compounds having high vapor pressures. These losses may be minimized through use of the Kuderna-Danish concentrator shown in figure 1 [7]. With this apparatus, solutions can be concentrated from several hundred milliliters to a few milliliters in one step with only small losses of even somewhat volatile compounds. This procedure has the added advantage of speed, requiring only 15-30 minutes to reduce a 200 ml volume of hexane to about 5 ml. Further concentration to 50 or 100 μ l is possible using micro-Kuderna-Danish concentrators. Often the use of a "keeper," usually a heavy paraffin oil, is desirable to assure minimal losses of more volatile compounds. The keeper, however, may interfere with some analyses (e.g., flame ionization



Figure 1. Kuderna-Danish concentrator with three-ball Snyder column and expansion flask.

of mass spectrometry). Some recovery data are given in table 2 for the concentration of a 100 ml hexane solution of several pesticides to 10 ml, 1 ml and 0.1 ml (the latter, with and without a keeper).

TABLE 2. *Losses of pesticides on evaporation in Kuderna-Danish concentrators*

Pesticide	Original amount in 100 ml hexane (μ g)	% Recovery on concentration to*			
		10 ml	1 ml	0.1 ml	0.1 ml with "keeper"
Diazinon	40	102 (2.4)	85 (4.4)	71 (1.8)	83 (1.8)
Aldrin	1.0	103 (2.8)	85 (4.0)	69 (1.2)	81 (0.6)
Malathion	40	85 (2.4)	91 (5.2)	77 (3.0)	88 (0.6)
Parathion	10	93 (4.4)	84 (4.0)	70 (1.2)	82 (2.4)
Dieldrin	2.5	103 (5.6)	92 (4.0)	78 (0.6)	90 (1.2)
<i>p,p'</i> -DDT	5.0	96 (9.2)	91 (5.6)	78 (3.0)	90 (2.4)

* Averages of six determinations for each pesticide. Standard deviations given in parentheses. Gentle stream of nitrogen used to assist concentration below 10 ml.

D. IDENTIFICATION

Identification is the most difficult step of trace organic analysis. Generally, the residue chemist knows what compound or group of compounds he is seeking, and attempts identification by comparing the unknown he has isolated from the sample to a known reference standard. Since gas chromatography is most often used in the determinative step, the most frequently used method of identification is by comparison of the gas chromatographic retention times of the standard and unknown under identical conditions. This method presents many possibilities for error as tens of thousands of organic compounds may elute from a given gas chromatographic column under a single set of conditions during the normal time span required for analysis. In addition, the retention time of the compound will change with fluctuations in carrier gas flow rate, column temperature and other parameters, as well as with variations in injection techniques. To circumvent some of these pitfalls, the analyst may use relative retention times; that is, he can compare the retention times of the unknown and standard to that of the third compound, which has been added to both the sample and standard solution. He may also compare relative retention times on two columns of different polarities (see fig. 2). This dual-column confirmation can be carried out in one operation if a column "combiner" is used. With some selectivity in the isolation and

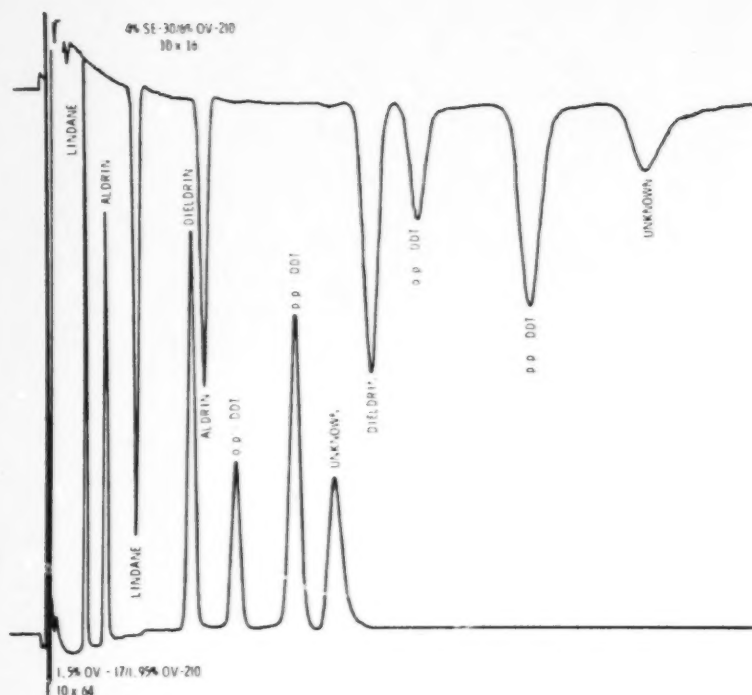


Figure 2. Dual column confirmation of pesticides by electron capture detection. Pesticides (from left): lindane, aldrin, dieldrin, o,p'-DDT, p,p'-DDT and unknown. Top chromatogram: 4% SE-30/6% OV-210 on Gas-Chrom Q. Bottom chromatogram: 1.5% OV-17/1.95% OV-210 on Gas-Chrom Q. Both columns 6.3 x 183 cm glass. Carrier gas: nitrogen, 65 ml/min. Oven temperature: 200 °C. Detector: tritium, D.C., 215°. Chart speed: 1.27 cm/min.

clean-up steps (*i.e.*, fractionation of compounds by class or functionality), the electron capture detector and dual-column confirmation can probably afford 80 to 90 percent qualitative accuracy for the determination of organic compounds with high electron affinities. Most laboratories do not conduct further confirmatory studies.

When it is possible to use two gas chromatographic detectors, further confidence in qualitative accuracy can be achieved. For example, simultaneous analysis by electron capture and flame photometric gas chromatography is very useful for confirmation of organophosphorous pesticides (see fig. 3). In the case of the phosphorothioate class of insecticides (which includes malathion and parathion), simultaneous chromatograms

can be generated by monitoring both phosphorus (POH) and sulfur (S_2) emissions with a dual flame photometric detector.

If more qualitative accuracy is to be achieved, more sophisticated means of identification are required. Spectroscopic analysis usually affords the most information about an organic compound. Infrared and nuclear magnetic resonance spectroscopy are widely used by the organic chemist for structure identification. However, even with Fourier transform systems to enhance the signal to noise ratios of these spectrometers, detectabilities are still in the microgram to high nanogram range. This precludes their use for most trace analyses. Furthermore, interfacing with a gas chromatograph is difficult or impossible. Mass spectrometers, on the other hand, are readily interfaced with gas chromatographs and in many cases have sufficient sensitivity for identification at the residue level. Achievable detectabilities are in the nanogram range for total ion monitor-

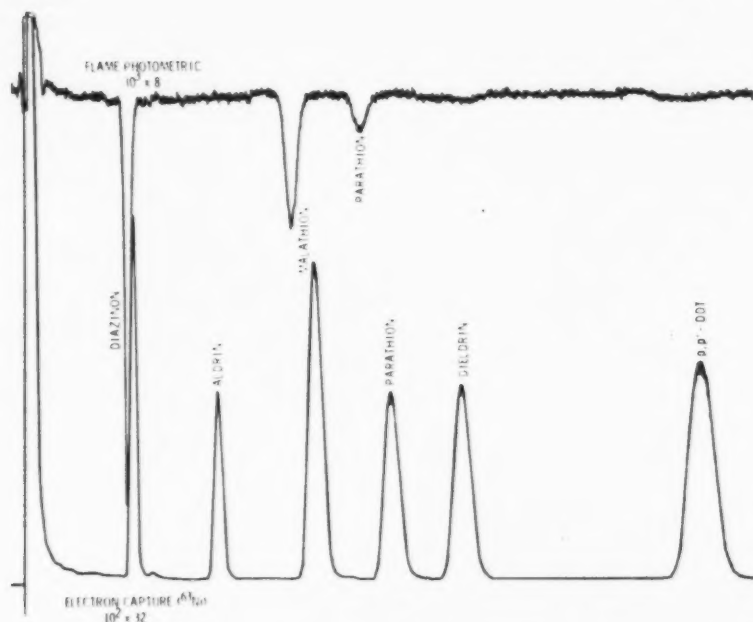


Figure 3. Simultaneous gas chromatograms of organochlorine and organophosphorus pesticides using electron capture and flame photometric detectors. Columns: 6.3×183 cm 4% SE-30/6% OV-210 on Gas Chrom Q, 200°, nitrogen carrier gas; Detectors: Pulsed Nickel-63, 270°; Flame photometric detector, 526 nm filter, 200°. Chart speed: 1.27 cm/min.

ing or as low as several picograms for single ion monitoring. Although the mass spectrometer is most often used as a qualitative instrument, it can also be very useful for quantitative trace organic analysis. This use is discussed in the following section.

E. QUANTITATIVE MEASUREMENT

The first prerequisite to accurate quantitative measurement is the availability of good *primary standards*. In organic analyses, this is not always easy to assure. Because of the large numbers of different compounds required, the analyst must depend mostly on the manufacturers or chemical specialty suppliers for reference materials. A purity level of 95 to 99 percent is generally considered adequate. All too often, however, the compound stated to have 98 percent purity by the supplier is, in fact, only 75 percent pure. To be safe, the chemist should purify the chemical by recrystallization, sublimation, distillation, or other suitable means. Differential thermal analysis (DTA) often is convenient for determining the purity of organic analytical standards.

Organic compounds are subject to a wide variety of oxidation, hydrolysis, isomerization and polymerization reactions. Instability of organic standards is, therefore, often a problem. Storage conditions should be such as to retard degradative processes; *e.g.*, storage under refrigeration, inert atmospheres and protection from light. Purity also should be checked periodically by melting point, DTA or other appropriate means.

Preparation and use of secondary standards also must be given due consideration. In multiclass, multiresidue analysis, working standards are made up with a number of components in one solution in order to reduce analysis times. Care must be exercised that these mixtures do not contain compounds which are incompatible. Stabilities of standard mixtures may differ from those of pure standards or solutions of individual primary standards.

In some cases single compounds may not exist for, or be representative of, the organic pollutants of interest. The chemical may be manufactured, used and introduced into the environment as a complex mixture, the exact composition of which is unknown. A well known example of this are the polychlorobiphenyls or PCB's. There are 210 possible isomers and homologs of PCB's, 102 of which are considered probable [8] and likely to be encountered in environmental samples. A commercial PCB mixture may vary slightly in composition from batch to batch, but the residue chemist's primary problem is trying to interpret what he finds in a sample in terms of the source of contamination. The distribution patterns of

PCB's are altered in the environment because of differences between the volatilities, solubilities and chemical reactivities of the individual components of the original mixture. If a PCB mixture is introduced into an environmental or biological medium and later extracted for analysis, the pattern of peaks determined by gas chromatography may be drastically altered from that of the "standard" (e.g., see fig. 4). For lack of a better way, quantification is usually accomplished by comparison of total peak heights or areas of all matching g.c. peaks in the sample with that for the standard. When the source of contamination is unknown, identification is also made by peak matching with commercial mixtures. Any similarity is likely to be purely fortuitous.

The problem of accuracy in PCB analysis is not easily soluble. Because of the complexity of commercial mixtures, identification of individual

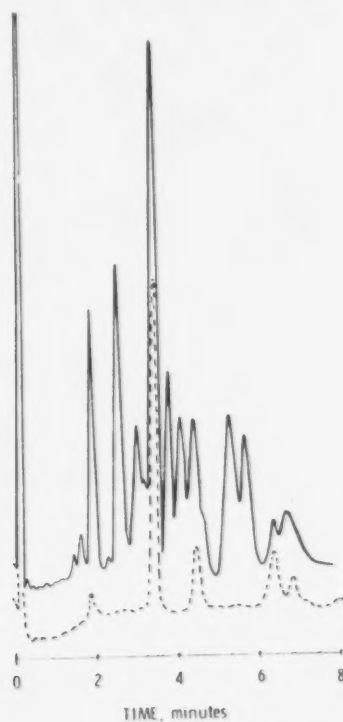


Figure 4. Alteration of polychlorobiphenyl mixture by rat. Top chromatogram: Aroclor 1016 standard. Bottom chromatogram: PCB's extracted from brain of rat dosed with Aroclor 1016 for 12 months.

isomers and homologs is not practical. Complete separation by gas chromatography is impossible and even the mass spectrometer cannot distinguish between isomers. One possible solution to the quantitative problem is to convert all of the PCB components to one entity by perchlorination [9]. The total PCB content is then measured as decachlorobiphenyl. Analytical methodology for this technique, however, has not been established completely.

It is of interest to note that before the existence of PCB's in the environment was recognized, pesticide residue chemists reported a PCB component as DDT in many samples. Doubtlessly, other PCB components have also been misidentified as organochlorine pesticides by residue chemists. The relative retention times (to aldrin) of the major components of a commercial PCB mixture are shown for comparison with those of several important pesticides in table 3. This particular mixture is no longer manufactured in the United States, but other mixtures of mostly lower molecular-weight PCB's are in use.

Similar problems associated with complex commercial mixtures exist in the pesticides field. Toxaphene, strobane, and chlordane are important examples. These mixtures are even more complex than PCB mixtures in that they consist not only of isomers and chlorine homologs of a given compound, but may also contain several structurally different com-

TABLE 3. *Relative retention times for polychlorobiphenyls and some pesticides*

Aroclor 1248	RRT _a *	Pesticide	RRT _a
	0.44	2,4-D (ME)	0.44
	.55	Simazine	.54
	.62	Lindane	.62
	.73		
	.80		
	.83	Heptachlor	.83
	.90	Ronnel	.90
	1.03		
	1.10		
	1.18		
	1.32	Methyl parathion	1.34
	1.50		
	1.80	<i>p,p'</i> -DDE	1.82
	1.92	Captan	1.92
	2.24		
	2.30		
	2.73	Endosulfan II	2.72
	3.12	<i>p,p'</i> -DDT	3.12

* RRT_a: Retention time relative to aldrin on 4 percent SE-30/6 percent QF-1 at 200°, 70 ml/min.

pounds. A gas chromatogram of technical chlordane is shown in figure 5. While only 18 different components of chlordane have been identified [10], one of these is heptachlor, a widely used insecticide. Some of the components of chlordane are readily oxidized, especially in biological systems, while others are unchanged. Identification and quantitation of chlordane in environmental samples is, therefore, nearly impossible. Metabolites such as oxychlordane [11,12] can be determined and used as an indication of chlordane exposure, but quantitative assessment of exposure or contamination is not possible.

Reference materials consisting of the compounds of interest at known concentrations in different substrates are required for determination of recoveries and limits of detection in multiresidue analysis. Preparation of accurate matrix standards may not be simple. The chemist must be able to disperse uniformly the standard compound in the matrix and remove residual solvents without loss of the compound. Even then, the nature and extent of the binding of the compound to the substrates may not be the

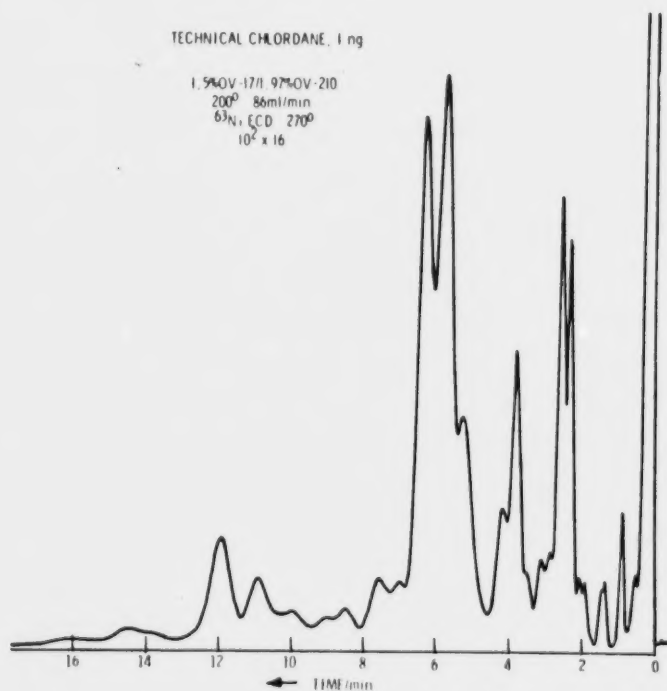


Figure 5. Gas chromatogram of technical chlordane.

same as that which would result through usual routes of environmental contamination or biological assimilation. Hence, use of recovery data in quantitative analysis still leaves an element of uncertainty. The analyst should be alerted to these potential problems and devote sufficient time to development of "spiking" techniques.

Preparation of the sample for analysis is also very important with respect to quantitative accuracy. Inefficient extraction and losses encountered in sample preparation are principal contributors to analytical errors in trace organic analysis. Steam distillation resulting from sample drying techniques can cause high losses, as can adsorption to drying agents.

Sample collection is frequently difficult to achieve with precision and accuracy. The collection of a representative sample has always been a major challenge to the environmental chemist. A great deal of study, involving population distribution, meteorology and statistics, goes into the design of monitoring operations conducted by the U.S. Environmental Protection Agency in efforts to establish accurate pollution profiles. Continuous air monitoring is probably the most difficult to accomplish accurately, especially for airborne organic pollutants. These compounds are usually present in ambient air at concentrations in the nanogram per cubic meter (ppt) range. A collection device which samples a relatively high volume of air is necessary to meet the required limits of detection. To be efficient, the sampler must entrap the atmospheric constituents quantitatively in a very short period of time and retain them against elution by the large volumes of air which subsequently or concurrently pass through the collection medium. Since organic compounds found in the atmosphere differ widely in their volatilities and chemical functionalities and may be present in gaseous, aerosol, particulate or particulate-adsorbed states, sampling efficiencies will necessarily vary considerably for any given sampler. The difficulty of collection of pesticides from air demonstrates this problem well. As a result, a satisfactory sampler for airborne pesticides has not yet been developed. In fact, a single air sampler for all pesticides never may be practical.

Calibration of air samplers is not always simple, particularly in the field, and is often neglected or improperly done. To determine sampling efficiencies, known concentrations of compounds in accurately measured air volumes must be generated then introduced into the sampler over both intermittent and continuous time sequences. This is difficult to achieve experimentally, hence, sampling errors probably out-weigh analytical errors in most cases.

After entrapment, the organic compounds must be extracted or otherwise removed from the sampling medium for analysis. In some cases it may be possible to elute them from the collection medium directly into the

analytical device by application of heat and gas flow. In most cases, however, this is not practical. If the compounds are to be retained effectively in or on the sampling medium after collection, they may not be removed easily for analysis. Since rather polar collection media are required, recovery of the compounds may be as difficult as recovery from fatty tissue and soil. Interferences from the multitude of airborne organic constituents, as well as those inherent in the collection medium, also contribute to the difficulty of qualitative and quantitative analysis.

Similar problems also apply to continuous water sampling. Batch sampling, either of water or air, increases the probability of obtaining non-representative samples. In addition, adsorption of trace quantities of the compound on container walls significantly can reduce accuracy. It has been estimated that the walls of most 1-liter sample containers have an adsorption capacity of 5×10^{-5} g [1]. Filling such a container with an air sample for analysis even at the part-per-million level (1.2×10^{-6} g) could, theoretically, result in complete loss by wall adsorption. Certainly a dynamic equilibrium will exist between already present adsorbates (e.g., water) and the compound of interest, but high losses are still possible. The importance of thorough flushing of the sample container is quite evident.

Many organic compounds are more susceptible to oxidation or other degradative processes when present in low concentration in a sample matrix. Delays between collection and analysis should, therefore, be minimized.

Contamination is another major enemy of quantitative and qualitative accuracy in trace organic analysis. Oils from finger tips; residue from improperly cleaned glassware; organic matter in distilled water; trace impurities in solvents, reagents, and chromatographic adsorbents; and extractables from plastics are all commonly encountered sources of contamination. Care must be exercised to avoid touching interior parts of glassware, including rims, even with gloved hands. Glassware must be meticulously cleaned and rinsed, before use, with solvents of high purity. Chromic acid cleaning is necessary for most glassware, especially for organic sample substrates. Water must be double- or triple-distilled and even then it must often be extracted prior to use. Solvents must be high purity. "Nanograde" or "pesticide" qualify usually suffice, but even these should be concentrated and analyzed for possible interfering contaminants before use. Anhydrous sodium sulfate has long been known to be a source of an unknown contaminant, which can interfere with pesticide residue analysis by electron capture gas chromatography. Extraction with hexane is necessary for purification of this drying agent. Phthalates and other plasticizers often show up in organic residue determinations. Sources of these may be plastic bottle caps, plastic sample containers and

gloves. Generally only fluorocarbons, such as Teflon, Tedlar, or Kel-F can be considered free of plasticizers and other extractable additives.

Detector response reproducibility is a factor frequently overlooked by the trace analyst in the assessment of accuracy. In electron capture gas chromatography, operation of the detector outside the limits of its linearity of response is probably the greatest single source of quantitative error. The EC detector has a relatively limited range of linearity. This is especially true for the nickel-63 detector. In an electron capture detector, the standing current generated by the radioactive cathode varies considerably with the applied voltage. A response curve such as curve A shown in figure 6 should be generated by the standing current when the polarizing voltage is varied. The standing current increases with voltage until a maximum or plateau is reached. The voltage at which this plateau is reached will depend on the condition of the detector. A "dirty" detector will require a higher polarizing voltage for maximum response (see curve B, fig. 6). The most nearly linear response is obtained immediately below the plateau (in region X, fig. 6). Sensitivity is generally greatest in this region also, but it may be more desirable to operate at a slightly higher voltage than that which produces maximum sensitivity in order to avoid the peak

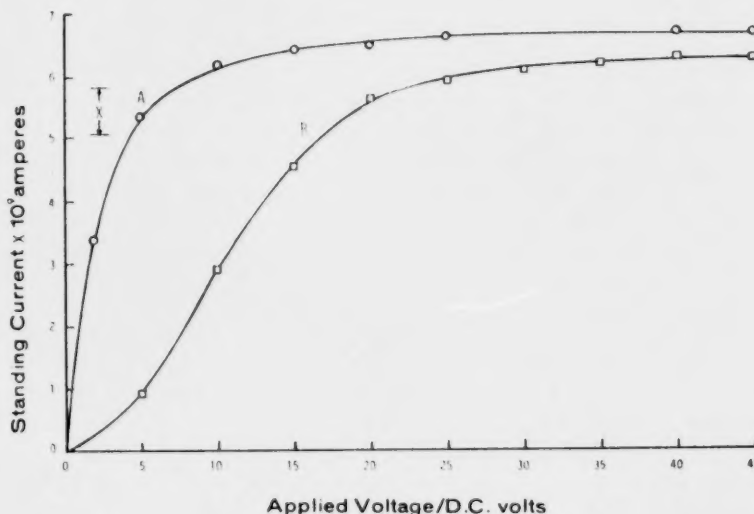


Figure 6. Standing current versus applied polarizing voltage dc for a tritium parallel-plate electron capture detector. Curve A: Clean detector (X = optimum range of operation). Curve B: Fouled detector.

"overshoot" shown in figure 7. Optimum performance is usually at 80 to 90 percent of maximum standing current. If one operates above 90 percent of the maximum response, unusual behavior can sometimes result and can lead to large and unsuspected quantitative errors. Figure 8A shows the response of an EC detector at the optimum polarizing voltage to a group of pesticides. In figure 8B, the response has changed drastically, producing elongated peak sides. This condition can be caused by in-

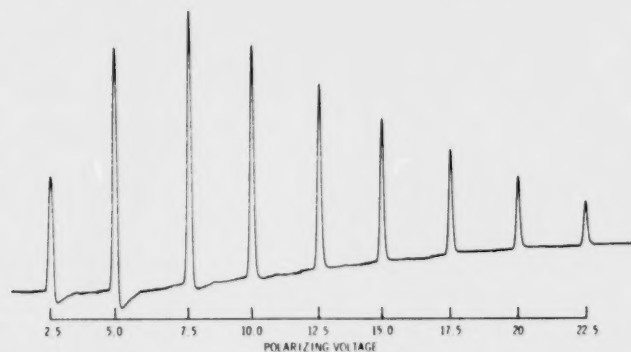


Figure 7. Electron capture response versus applied voltage dc for aldrin. Tritium parallel-plate detector. Optimum voltage is 10 volts dc.

creasing the standing current to above 90 percent maximum. Sometimes the standing current can increase due to the cleansing action of solvents or heat on the detector. This can occasionally happen during the course of a routine analytical procedure, as is illustrated by the two gas chromatograms shown in figure 9. In this case, the analyst was unaware that anything was wrong since the effect was obscured by the narrow peak profile [13].

Many of the problems discussed above can be avoided by pulsing the polarizing voltage (*i.e.*, using alternating rather than direct applied voltage). However, the linear dynamic range of the detector may be decreased in this mode.

Other gas chromatographic detectors have peculiar response behavior or linearity problems. A thorough understanding of the detector operation and close control over its operating parameters are prerequisites to attaining accurate quantitative results in gas chromatographic analysis.

Column performance must also be considered with regard to the overall precision and accuracy of gas chromatographic analyses. Poor g.c. column performance can result in inefficient resolution of compounds,

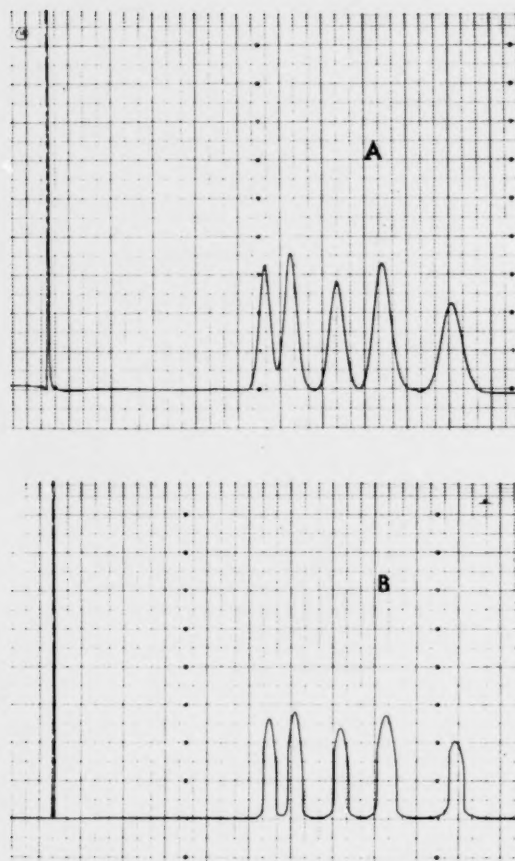


Figure 8. Trace A: Gas chromatogram of p,p'-DDE, o,p'-DDD, p,p'-DDD and p,p'-DDT; tritium electron capture detector, dc mode, optimized polarizing voltage. Trace B: Same as A except at elevated polarizing voltage (standing current above 90% of maximum).

skewed peaks from which it is difficult to obtain accurate quantitation, depressed detector sensitivity and poor recoveries.

Stationary phases for g.c. analysis must be carefully selected to satisfy the operating conditions required. When elevated temperatures ($> 200^{\circ}$) are necessary, "column bleed," or loss of the stationary liquid phase from the column substrate, may occur and interfere with the detector response or sensitivity. This is particularly true where tritium electron capture detectors are being used. The upper limit of operation for a ^3H detector is set

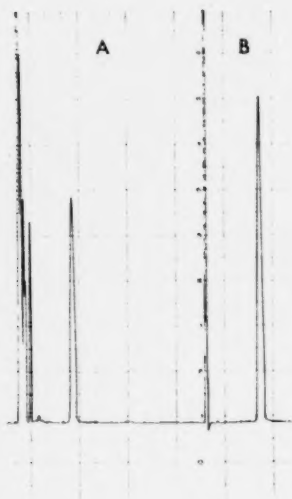


Figure 9. Trace A: Gas chromatogram of 2,4-D methyl ester using tritium electron capture detector at optimum polarizing voltage. Trace B: Same as A but with elevated polarizing voltage.

by the Atomic Energy Commission at 220°. Temperatures of 200° or more are necessary to achieve practical elution times for most complex organic compounds. With such small temperature differentials between column and detector temperatures, condensation of stationary phase in the detector can rapidly destroy its sensitivity.

Proper preconditioning, silylation and priming of a column are necessary to assure complete and uniform elution of compounds from the column and to reduce thermal degradation during chromatography. Degradation of column performance can also result from accumulation of oils and other high boiling residues. Figure 10 shows the deterioration in performance of a g.c. column with age as manifested by the dehydrochlorination of p,p'-DDT (peak 3) to p,p'-DDD (peak 4).

The efficiency of a g.c. column is also affected by the rate of flow of the carrier gas, as is shown in table 4. Once an optimum flow rate is established, care must be taken to maintain it.

Acquisition and interpretation of data are the final steps in qualitative and quantitative analyses. How this is done can in large measure affect accuracy. In gas chromatographic analysis, detector response is usually measured from recorder plots or chromatograms. The chromatographer usually employs one of three means of quantifying chromatographic

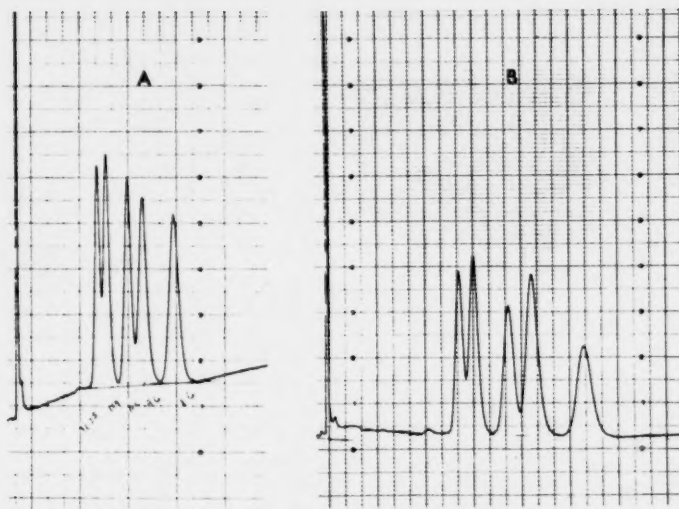


Figure 10. Gas chromatograms of p,p'-DDT, o,p'-DDD, o,p'-DDT, p,p'-DDD and p,p'-DDT on 4% SE-30/6% QF-1 at 180 °C. Trace A: New column; Trace B: Heavily used column upon which p,p'-DDT is converted to p,p'-DDD.

TABLE 4. Effect of carrier gas flow rate on column efficiency in gas chromatography

Flow rate (ml/min)	Theoretical plates (N)
20	1,500
30	1,970
45	2,140
60	2,050
92	1,590
145	1,310

peaks: (a) direct measurement of peak height; (b) manual estimation of peak area; or (c) electronic integration of peak area. Although most chemists tend to think of the last method as the most accurate, this is not always the case. For tall, slender peaks as type A in figure 11, peak height measurement is as accurate as any method and is the most convenient method to use. When broader peaks such as types B and C in figure 11 are involved, area measurements are generally more accurate. However, even in these cases peak heights may be used accurately if direct comparison is made to a standard of the same compound at about the same

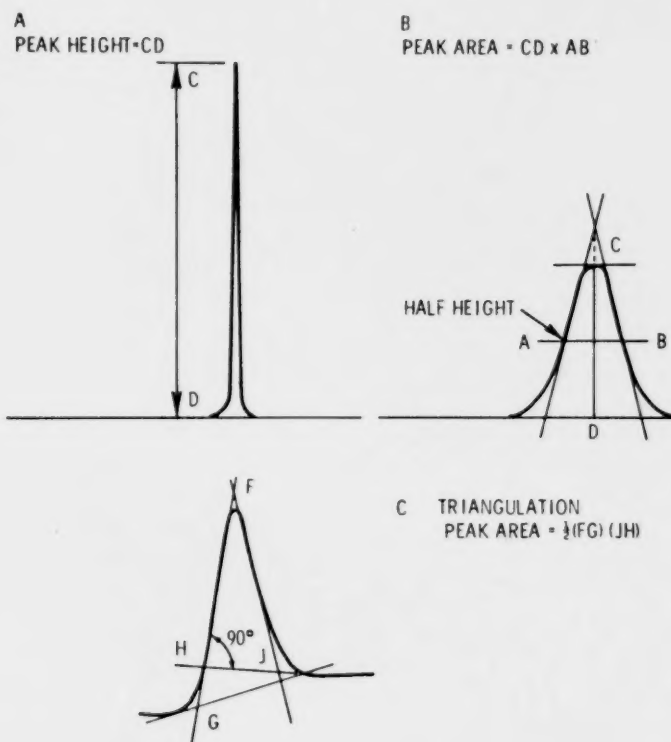


Figure 11. Three types of chromatographic peaks and appropriate methods of estimation for quantitative analysis.

concentration. It is good practice in any case to maintain standard and unknown peak heights (or areas) within 25 percent of each other. Electronic integration affords the highest precision when the peaks are well resolved and the baseline is stable. In a recent study of accuracy and precision in gas chromatography, Grant and Clark [14] found that use of peak height measurements provided comparable precision to that of an electronic integrator and was less affected by long-term variations. They also concluded that the product of peak height and width at half height method of manual area approximation (see type B, fig. 11) gave more accurate results than triangulation (see type C, fig. 11).

In comparing g.c. peaks of unknowns and standards several other precautions should be exercised. The standard and unknown should be analyzed within the shortest possible time span to avoid changes in

column conditions (temperature and flow rate). The standard should be injected immediately before and after the sample whenever practical. The same injection volume and type of solvent should be used for both sample and standard. Detector parameters (sensitivity settings) should be maintained constant throughout the analysis.

As in any analytical procedure, repeated analysis and statistical treatment of data for the rejection of outliers should improve accuracy. However, when large numbers of samples and/or long analysis times are required, this is difficult to accomplish in the available time. Automatic sampling systems with associated computerized data acquisition and reduction systems may partially relieve the burden of repetitive analysis. However, such systems seldom are capable of carrying out the most time-consuming aspects of organic analyses; *i.e.*, extraction, isolation and clean-up. Some success with automated clean-up operations utilizing such techniques as sweep co-distillation [15] and gel permeation chromatography [6] have been reported.

While *mass spectrometry* more frequently serves the trace organic analyst in qualitative analysis, it also can, and often does, satisfy his quantitative analytical needs. Both quantitative and qualitative accuracy can be increased by techniques such as mass fragmentography, isotope dilution, field desorption and chemical ionization. By these means detectabilities can be extended into the middle to low picogram range for many compounds.

In mass fragmentography [16], one or more specific ions in the mass spectrum are monitored selectively and simultaneously. This technique, also known as multiple ion detection (MID) or programmable multiple ion monitoring (PROMIM), will permit several ions from a single compound to be detected and accurately compared. Coeluting compounds from a gas chromatograph can also be simultaneously and quantitatively measured by MID. Sensitivity is increased by about two orders of magnitude over total ion monitoring. The utility of MID has been demonstrated for quantitative trace analysis of pesticides [17,18] and of drugs and drug metabolites [19-21]. Quadrupole mass spectrometers are better suited for MID than magnetic instruments because more rapid switching between ions is possible.

Double beam mass spectrometers allow separate recording of both reference and unknown compounds simultaneously, which increases the accuracy of mass measurement. Such instruments are very expensive, however, and are less popular.

Isotope dilution provides for quantification of organic compounds when complete isolation is not possible. In this procedure, the sample is "enriched" by the addition of a known quantity of the suspected compound

which has been labeled with a stable isotope (*e.g.*, ^{37}Cl). The isotopic abundances are determined from the mass spectra and the amount of compound (W) is calculated from the equation:

$$W = W_L(A/A_L - 1),$$

where W_L is the amount of labeled compound added and A and A_L are the abundances of the total and label isotope, respectively. The method suffers from poor precision (± 1 to 2%). However, by combined isotope dilution and MID techniques precision of ± 0.1 to 1 percent and accuracies of ± 0.1 to 3 percent have been reported [22].

Chemical ionization mass spectrometry [25] probably has more utility in structure elucidation than in quantitative analysis. Although a fragmentation mechanism is employed, high abundances of quasi-molecular ions are produced, which facilitates both identification and quantification. This technique has been found useful for polychlorobiphenyls [26,27] and drug analysis [28].

Special computer techniques such as signal averaging have been employed to increase signal-to-noise ratios in trace mass spectroscopic analysis for improved accuracy [29]. In signal averaging, many successive scans are made over a narrow mass range and are then added together to produce an integrated mass spectrum. By this procedure, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin has been quantified in biological media at levels down to a few picograms [30].

Combined gas chromatography-mass spectroscopy has also made possible the determination of benzo(a)pyrene and benzo(e)pyrene in ambient air samples [31].

F. QUALITY CONTROL

The last and one of the most important requisites for accuracy in trace organic analyses is the maintenance of a good quality control program. This is particularly important when many routine analyses are conducted and when more than one laboratory is involved in the analyses.

Quality control (QC) can be classified into two types: intralaboratory and interlaboratory. The former is concerned with establishing and maintaining high levels of precision and accuracy within the laboratory. An intralaboratory quality control program provides for optimization and standardization of analytical methodologies and instrumentation, and systematic analysis of standards and blanks for maintenance of precision and accuracy. Interlaboratory quality control is necessary when two or more laboratories are participating in the analysis of pooled or related samples.

In the latter program, a central monitoring laboratory is responsible for (a) the establishment and implementation of standard methodologies, (b) the provision of standard reference materials and collaborative check samples, and (c) the detection and correction of problems affecting the quality of output from participating laboratories.

In monitoring and regulatory agencies such as the U.S. Environmental Protection Agency and the Food and Drug Administration, the importance of effective quality control programs is obvious. The Pesticides and Toxic Substances Effects Laboratory (PTSEL) of the EPA and its predecessors has operated inter- and intra-laboratory quality control programs for nearly 7 years [32].¹ The program is designed to optimize the precision and accuracy of trace organic analysis conducted by contract laboratories and by the 10 EPA Regional Offices. Under this QC program is the National Human Monitoring Network.

The Laboratory has developed and is continually improving analytical methodologies for use by the participants. The methods are published in manual form [33]. Additionally, PTSEL operates a repository of over 500 organic chemical standards, with principal emphasis on pesticides. The Laboratory purchases under contract, pretests, and distributes gas chromatographic and column chromatographic packing materials. Electronic maintenance, calibration and trouble-shooting service for gas chromatographs and ancillary detector systems is also provided. Protocols for preventative maintenance of instrumentation (which is mostly standardized) and for acquisition and evaluation of all solvents and reagents have been established under the intralaboratory program.

Intralaboratory standards of pesticides in adipose tissue (rendered chicken fat) and human blood and interlaboratory check samples are prepared by PTSEL staff and distributed on prescheduled bases to each participating laboratory. Each participant is graded on both qualitative and quantitative accuracy, with greater weight given the former.

Over the years of operation of this quality control program considerable improvement in precision and accuracy has been achieved. Mean relative standard deviations for determination of multicomponent mixtures of pesticides in fat and blood samples have decreased from 36 to 38 percent in 1968 to 12 to 16 percent in 1974 (see table 5). Other quality control programs for organic trace analysis within EPA are administered by the Methods Development and Quality Assurance Research Laboratory and the EPA Mass Spectrometer Users Group.

Most needed in this and other quality control programs for trace organic analysis, are standard reference materials of many organics in such

¹A training program consisting of seven courses covering the complete analytical and instrumental methodology was offered from 1966 until 1972, when operating funds were terminated.

Other contributors to qualitative errors are sample contamination and chemical alteration before or during analysis.

More sensitive, specific analytical instruments are greatly needed in trace organic analysis. Mass spectrometry satisfies this need to a large extent at present.

An effective quality control program is prerequisite to both qualitative and quantitative accuracy. Good standard reference materials should also be made more available to the trace organic analytical chemist.

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